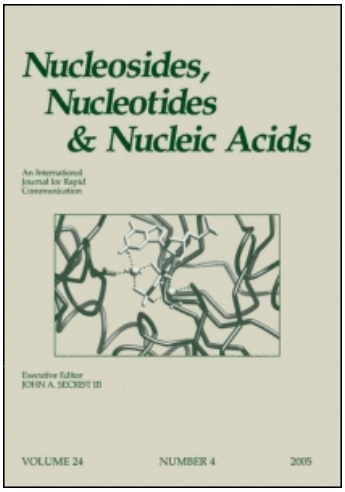


Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

New Potent and Selective Inhibitors of Herpes Simplex Virus Tbyhidine Kinase

J. A. Martin[a]; I. B. Duncan[a]; M. J. Hall[a]; P. Wong-Kai-In[a]; R. W. Lambert[a]; G. J. Thomas[a]
[a] Research Division, Roche Products Limited, Hertfordshire, England

To cite this Article Martin, J. A. , Duncan, I. B. , Hall, M. J. , Wong-Kai-In, P. , Lambert, R. W. and Thomas, G. J.(1989) 'New Potent and Selective Inhibitors of Herpes Simplex Virus Tbyhidine Kinase', Nucleosides, Nucleotides and Nucleic Acids, 8: 5, 753 — 764
To link to this Article: DOI: 10.1080/07328318908054213
URL: http://dx.doi.org/10.1080/07328318908054213

NUCLEOSIDES & NUCLEOTIDES, 8(5&6), 753-764 (1989)

NEW POTENT AND SELECTIVE INHIBITORS OF HERPES SIMPLEX VIRUS THYMIDINE KINASE

J A Martin*, I B Duncan, M J Hall,
P Wong-Kai-In, R W Lambert, and G J Thomas

Research Division, Roche Products Limited,
P O Box 8, Welwyn Garden City,
Hertfordshire AL7 3AY, England

Abstract. Analogues of 5-ethyl-2'-deoxyuridine with modifications in the 5'-position have been prepared as potent inhibitors of herpes simplex virus thymidine kinase (HSV TK). The most potent compound in the series is extremely selective for the viral enzyme, antagonises the antiviral activity of acyclovir *in vitro* and shows a protective effect in virus-infected mice.

Over the past decade there has been a growing interest in the identification of herpes simplex virus (HSV) genes that are involved in neurovirulence, latency and reactivation and it is now clear that in addition to host cell elements, several viral gene products may be involved in HSV pathogenesis. These include $\alpha 22$,[1] thymidine kinase (TK)[2,3,4] and other as yet unidentified genes located in the regions of 0.71-0.83 and 0.25-0.52 map units.[5,6] These genes may restrict replication or spread of virions to different sites within the nervous tissue such as the sensory ganglia, the spinal chord or the brain but it is not clear whether multiple mutations may act in a co-operatively restrictive manner. The situation is further complicated by studies that have shown that the host genotype also plays a significant role in susceptibility to latency and CNS spread of herpes simplex virus.[7] Clearly, such studies have a profound bearing on our understanding of herpes virus reactivation and recrudescence and may eventually provide avenues for new therapeutic strategies for controlling potentially serious human diseases such as herpes genitalis, herpetic keratitis and herpetic encephalitis.

Only in the case of TK, however, has it proved possible to ascribe a biochemical function to the gene product, namely the enzymic phosphorylation of thymidine to thymidylic acid (TMP) and thymidine diphosphate; deoxycytidine is also a substrate for the viral enzyme but not for its cellular counterpart. Herpes simplex virus was first shown by Kit and Dubbs[8] to induce a novel TK as long ago as 1963, and hints that thymidine kinase defective (TK^-) mutants might have modified virulence arose from studies in the rabbit using an isolate obtained by culture in IUdR-containing medium.[9] However, the mutation in this isolate was not mapped at the time and it was not until 1978 that Field and Wildy[2] showed unambiguously that proven TK^- mutants of both HSV-1 and HSV-2 were less virulent in mice when inoculated into both central and peripheral sites. These observations have been repeatedly confirmed in model infections of the genital tract,[10] the eye,[11] the ear[2] and other sites but there is less agreement regarding the ability of TK^- mutants to establish latency. Some workers have revealed an apparent correlation between the extent of TK synthesis and a propensity to cause latent infection.[12,13,14] In a number of these studies, however, the data are difficult to interpret as TK^- point mutants were employed. These have a back-mutation rate perhaps as high as 1 in 1000, and the inocula were undoubtedly mixed, which in principle permits *in vivo* complementation.[15,16]

An alternative approach to investigate the role of TK in herpes infections would be through potent and specific inhibitors of the viral enzyme.

Design of Inhibitors

The process catalysed by TK is illustrated in Figure 1. Thymidine is converted to its monophosphate with adenosine triphosphate (ATP) as the phosphate donor, a process that requires the presence of a divalent cation such as magnesium. The chemical transformations that occur suggest various approaches to the design of inhibitors based on either substrate analogues, metal chelation or product analogues. A vast number of substrate analogues have been

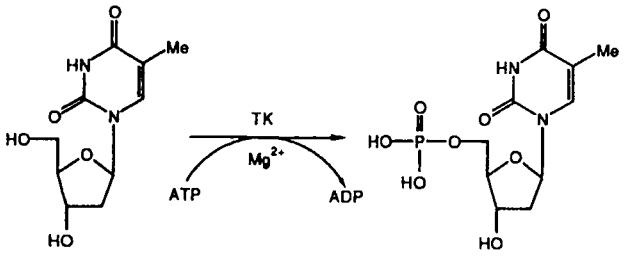


Figure 1

synthesised, many of which have good antiviral properties and are competitive inhibitors of TK with respect to the natural substrate although their antiviral activity is mediated as their triphosphates against the virus-coded DNA polymerase. The triphosphates themselves may elicit undesirable toxicities by interference with cellular polymerases, by incorporation into host cell DNA or through disturbance of cellular nucleotide pool levels. An example of such a poorly-selective compound is idoxuridine. The chelation of metal ions as an approach to TK inhibition has not been successfully exploited thus far. From a chemical standpoint the chelation of specific metals can be achieved but the targeting of these agents to specific sites has not been accomplished successfully, and indeed it is reasonable to suppose that such an approach would interfere with essential cellular processes, resulting in the appearance of side-effects. Thus we decided to pursue an alternative strategy by designing specific inhibitors of viral TK based on product analogues. We reasoned that this type of inhibitor would not be removed from the system by processes such as phosphorylation and would have the prospect of high specificity, a sustained inhibition and low potential for toxicity.

At the commencement of our work potent inhibitors of HSV TK were not known and we decided to prepare isosteric and isoelectronic analogues of thymidine monophosphate: some examples of the classes of compounds that we have made are shown in Figure 2. Encouragement for

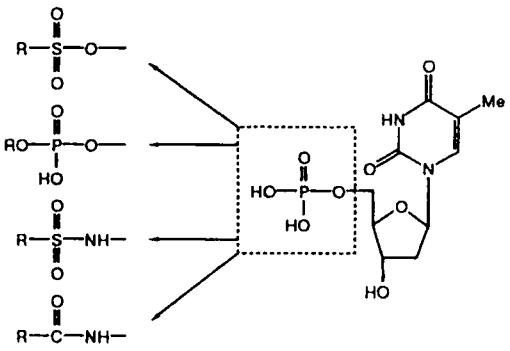


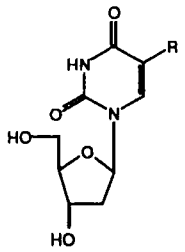
Figure 2

this approach came from studies designed to improve the antiviral efficacy of 5'-amino-2',5'-dideoxy-5-iodouridine through the preparation of N-acyl and N-sulphonyl derivatives[17] as prodrugs. The N-acyl derivatives were not antiviral *in vitro*, failed to enhance the potency of the parent nucleoside but did show weak inhibition of HSV-1 TK.

Our first concern was to address the problem of specificity towards the viral enzyme. We set out to identify as our starting material a nucleoside that would provide the desired selectivity, by having a high affinity for the viral enzyme and a low affinity for its cellular counterpart. Fortunately, relevant data were available from the literature[18] as set out in Table 1.

Idoxuridine, **1**, shows a high affinity for both the viral and cellular enzymes and consequently has a poor selectivity. Although trifluorothymidine, **2**, has high affinity for the viral enzymes it too has poor selectivity, particularly concerning the cellular cytosol enzyme, whereas 5-vinyl-2'-deoxyuridine, **4**, has high affinity for the cellular mitochondrial enzyme. Both 5-ethyl-2'-deoxyuridine, **3**, and 5-n-butyl-2'-deoxyuridine, **5**, show good potency and selectivity towards the viral enzyme. Based on these data we chose **3** as the nucleoside moiety most likely to provide potency and selectivity towards HSV-2 TK.

Table 1 - Dissociation Constants of Nucleoside Analogues

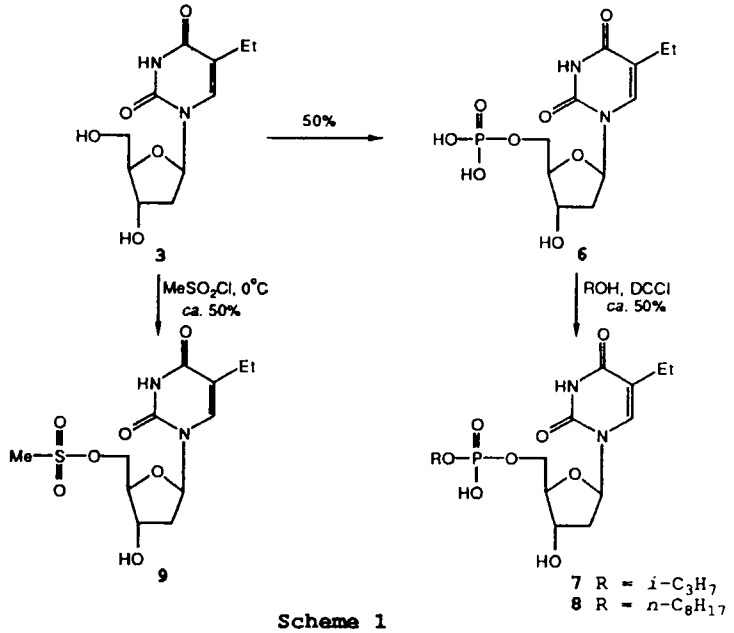


Compound	R	Dissociation Constant [μM]			
		Viral TK		cellular TK	
		HSV-1	HSV-2	cytosol	mitochondrial
1	I	0.6	0.3	7.4	8.2
2	CF_3	0.4	0.5	4.2	30
3	C_2H_5	0.7	0.3	82	30
4	$CH=CH_2$	0.5	0.5	35	1.7
5	$n\text{-}C_4H_9$	1.6	4.0	100	40

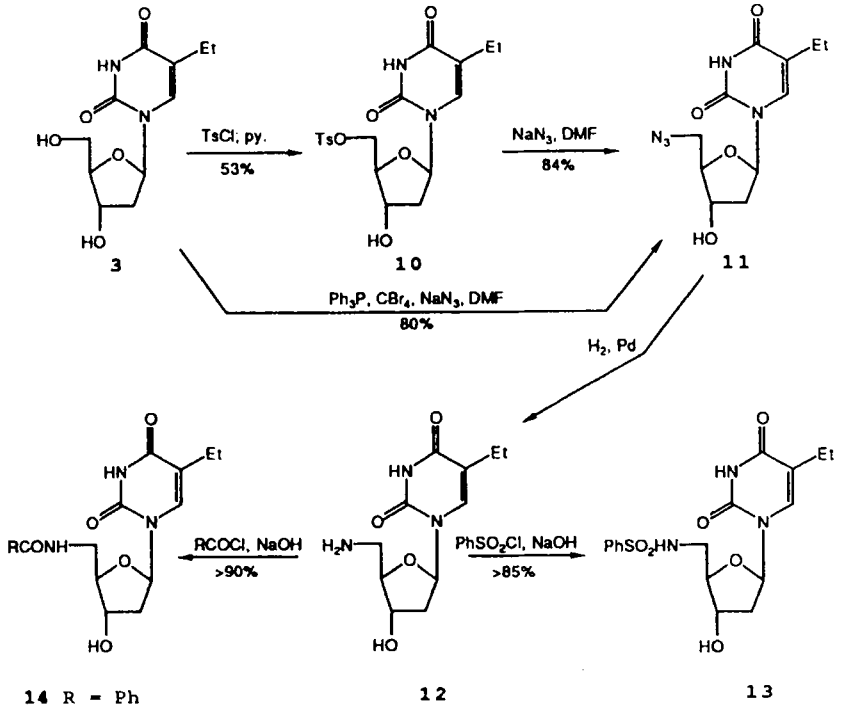
Chemistry

The starting material for all of the target compounds that we have prepared thus far is **3**, the most convenient synthesis of which is based on the procedure described by Robins.[19] The first of our target compounds to be synthesised were the phosphate and sulphonate esters shown in Scheme 1.

The monophosphate **6** was prepared using the procedure described by Tener.[20] Treatment of **6** with DCCI and either isopropanol or n-octanol in pyridine[21] gave compounds **7** and **8** respectively. The sulphonate **9** was readily prepared from **3** by treatment with methane sulphonyl chloride in pyridine solution using standard procedures.[22] Amides and sulphonamides were prepared from 5'-amino-2',5'-dideoxy-5-ethyluridine **12** as shown in Scheme 2. Thus, treatment of **3** with p-toluenesulphonyl



Scheme 1



Scheme 2

chloride gave the tosylate **10**. Displacement of the tosyl group with sodium azide using a modification of the literature procedures[22,23] gave the azide **11** in 45% overall yield. Reduction of the azide with hydrogen in the presence of palladium on carbon gave **12**. Later we found that the azide **11** is more directly accessible in a one-pot reaction using a modification of the procedure described by Yamamoto.[24] Typically in this reaction we use triphenylphosphine (1 eq.), carbon tetrabromide (1 eq.) and sodium azide (10 eq.) in dimethylformamide at room temperature overnight, a procedure that consistently gives **11** in greater than 80% yield.

Acylation of the amine **12** with appropriate acid chlorides using Schotten-Baumann conditions gives the amides **14, 15, 16, 17, 18** and **19** in almost quantitative yield. Similarly, treatment of **12** with benzenesulphonyl chloride gives the sulphonamide **13** in good yield.

Biological Results

Initially we examined the ability of each of the target compounds to inhibit TK from both HSV-1 and HSV-2. The viral enzymes were obtained from confluent monolayers of BHK[TK^-] cells infected with HSV-1 [HFEM] or HSV-2 [3345] at a multiplicity of 2-5 pfu's per cell and partially purified by column chromatography on DE52 cellulose. The cytoplasmic enzymes from HeLa and Vero cells were partially purified in a similar manner. The assay for measuring inhibition of TK was conducted as described by Honess.[25]

From the results in Table 2 it can be seen that all of the inhibitors except **14** are more potent against TK isolated from HSV-2 than against that from HSV-1, the difference being largest with the most potent inhibitor, compound **19**. The n-octyl ester **8** is markedly more potent than the isopropyl derivative **7**. This suggested to us that there may be a lipophilic binding region on the enzyme close to the 5'-position of the bound inhibitor. A further indication of this was seen, albeit to a lesser extent against HSV-2 TK, with the sulphonates **9** and **10**. We decided to exploit this effect in our search for even more potent inhibitors. Interestingly, the

Table 2 - Inhibition of Viral TK

Compound	IC_{50} [μM] HSV-1	HSV-2
7	208	40
8	9.2	1.5
9	8.1	4.8
10	12.7	4.1
13	15.2	4.6
14	3.1	3.2
15	1.0	0.3
16	1.7	0.8
17	0.7	0.28
18	0.18	0.07
19	0.03	0.004

Assay procedure :- 50mM Tris/HCl pH 8.0, 5mM ATP, 5mM $MgCl_2$, 0.33μM [3H]thymidine, 0.5mg/ml BSA and 50μl enzyme in a total volume 100μl. Incubation for 30 mins at 37°C.

sulphonate **10**, the sulphonamide **13** and the benzamide **14** have very similar potencies. The homologous amides **15** and **16** are more potent, particularly against the HSV-2 enzyme, probably indicating that the phenyl group in these derivatives achieves tighter binding. Potency with the phenoxyacetamide **17** is improved, a trend even more in evidence in compound **18**. The introduction of lipophilic substituents into the phenyl ring as in compound **19** further dramatically enhanced the potency, thus affording an inhibitor almost 1000 times more potent than the starting amide structure.

Having identified **19** as a very potent inhibitor of HSV-2 TK the biology of this compound was explored in more detail. First, the activity against the cytoplasmic enzyme from two mammalian cell lines was measured (Table 3) indicating that compound **19** shows remarkable selectivity (>50,000) for the viral enzyme. Secondly, the activity of these potent inhibitors is not due to a prodrug effect since the

Table 3 - Inhibition of Viral and Cellular TK

	IC_{50} [μM]			
Compound	HSV-1	HSV-2	Hela	Vero
3	0.34	0.51	36	41
12	3.1	18.8	51	88
19	0.03	0.004	>200	>200

Table 4 - Antagonism of ACV in vitro

Antagonist [10μg/ml]	ACV IC_{50} [μg/ml]	IC_{50} increase
none	0.31	
19	10	32X
Thymidine	10	32X

IC_{50} determined by plaque reduction assay using HSV-2 [3345] on Vero cells.

parent nucleosides **3** and **12** (Table 3) show markedly weaker enzyme inhibition, and very poor selectivity for the viral enzymes.

At this point further studies were conducted to elucidate aspects of the mechanism of inhibition shown by **19**. The kinetics of inhibition show that **19** is competitive with respect to thymidine and non-competitive with respect to ATP. From this result it is concluded that **19** locates, as expected, in the thymidine binding site and that there is no additional contribution to potency from the phenyl group occupying the ATP binding site.

As expected, compound **19** does not show antiviral activity in a plaque reduction assay since it is accepted that viral TK is redundant

Table 5 - In vivo Activity in Mouse

compound	dose mg/kg	survivors day 14
none	-	0/10
ACV	15	9/10
19	10	8/10
19	5	3/10

Balb/c mice (female, 5-6 weeks old). Infected by the i.p. route with 10^4 pfu of HSV-2 [3345]. Compound administered i.p. twice daily for 5 days starting 2 hours post infection.

for growth under these conditions. However, it does show a marked antagonism (32X) of the *in vitro* antiviral activity of acyclovir (ACV), Table 4. The same antagonism of ACV is observed with thymidine. Finally compound **19** was evaluated *in vivo*.

The data in Table 5 show that compound **19** does produce a protective effect in virus-infected mice. However, we have found that this activity is variable and is particularly sensitive to the strain of mouse, virus inoculum and formulation of the test compound. Metabolic studies into the *in vivo* fate of TK inhibitors and further antiviral evaluation in other animal models are currently taking place.

To summarise, we have designed compounds that are potent and highly selective inhibitors of HSV TK and have found that the most potent compound, although not intrinsically antiviral *in vitro*, antagonised the antiviral activity of ACV in a plaque reduction assay. The most potent inhibitor of HSV TK has shown a protective effect in the mouse under some conditions. Our initial results with these compounds have encouraged us to examine these and related inhibitors

in other biological systems and we are in the process of synthesising related compounds that might have improved properties.

ACKNOWLEDGEMENTS

We express appreciation to Mr D J Bushnell, Mr S J Dunsdon, Mr A C Freeman, Mr R A Hopkins, Mrs E Keech and Mrs H S Simmonite for their assistance in the preparaton of these compounds and to Mrs S L Beck, Mrs V M Gibson, Miss E T McHugh, Mrs P N Miller and Mrs A J Ritchie for obtaining biological data. We are also grateful to Dr R W Honess for providing the virus strains HSV-1 [HFEM] and HSV-2 [3345] and Dr H J Field for the BHK [TK$^-$] cell line.

REFERENCES

1. Sears A E, Halliburton I W, Meignier B, Silver S and Roizman B. J.Virol., **1985**, 55, 338.
2. Field H J and Wildy P. J. Hyg., **1978**, 81, 267.
3. Tenser R B and Dunstan M E. Virology, **1979**, 99, 417.
4. Sears A E, Meignier B and Roizman B. J. Virol., **1985**, 55, 410.
5. Thompson R L, Wagner E K and Stevens J G. Virology, **1983**, 131, 180.
6. Thompson R L, Cook M L, Devi-Rao G B, Wagner E K and Stevens J G. J. Virol., **1986**, 58, 203.
7. Kastrukoff L F, Lau A S and Puterman M L. J. Gen. Virol., **1986**, 67, 613.
8. Kit S and Dubbs D R. Biochem. Biophys. Res. Comm., **1963**, 11, 55.
9. Marcialis M A, La Colla P, Schivo M L, Flore O, Firinu A and Loddo B. Experiencia, **1975**, 31, 502.
10. Stanberry L R, Kit S and Myers M G. J. Virol., **1985**, 55, 322.
11. Gordon Y J, Armstrong D Sr., Brown S I and Becker Y. Am. J. Ophthalmol., **1983**, 95, 175.
12. Gordon Y, Gilden D H, Shtram Y, Asher Y, Tabor E, Wellish M, Snipper D, Hadar J and Becker Y. Arch. Virol., **1983**, 76, 39.
13. Tenser R B, Jones J C and Ressel S J. J. Inf. Dis., **1985**, 151, 548.

14. Tenser R B, Ressel S and Dunstan M E. Virology, **1981**, 112, 328.

15. Yamamoto H, Walz M A and Notkins A L. Virology, **1977**, 76, 866.

16. Tenser R B and Edris W A. J. Virol., **1987**, 61, 2171.

17. Markham A F, Newton C R, Porter R A and Sim I S. Antiviral Res., **1982**, 2, 319.

18. Cheng Y C. Antimetabolites in Biochemistry, Biology and Medicine, Ed. by Skoda J and Langen P (Pergamon Press), p.263, **1979**.

19. Robins M J and Barr P J. J. Org. Chem., **1983**, 48, 1854.

20. Tener G M. J. Amer. Chem. Soc., **1961**, 83, 159.

21. Khorana H G. J.Amer. Chem. Soc., **1959**, 81, 1959.

22. Lin T S, Neenan J P, Cheng Y C and Prusoff W H. J. Med. Chem., **1976**, 19, 495.

23. Horwitz J P, Tomson A J, Urbanski J A and Chua J. J. Org. Chem., **1962**, 27, 3045.

24. Yamamoto I, Sekine M and Hata T. J. Chem. Soc. Perkin 1, 1980, 306.

25. Honess R W, O'Hare P and Young D. J. Gen. Virol., **1982**, 58, 237.